Enantioselective Syntheses of Potent Retinoid X Receptor Ligands: Differential Biological Activities of Individual Antipodes

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The synthesis and characterization of chiral RXR selective ligands are described. The enantiomeric acids **2** and **3** were synthesized employing an enantioselective cyclopropanation procedure as the key step. Compound **2**, with an *S,S* configuration at C-9 and C-10, is a potent RXR agonist devoid of any RAR activity. The *R,R* enantiomer **3** is a weak RXR agonist and has demonstrable RAR activity in the receptor transactivation assays. The potent RXR activity of **2** was further confirmed in a hyperglycemic animal model (*db/db* mice). Compound **2** lowered glucose by 50% by day 7 at 2 mg/kg, whereas **3** had no effect at the same dosage. This further supports the contention that RXR mediated gene transcription is involved in the antidiabetic effects of RXR ligands.

Retinoids are small molecule hormones,[1] which exert their biological effects by activating nuclear receptors and modulating gene transcription. Retinoids play key roles in both embryogenesis and maintenance of various cellular processes such as cell growth and differentiation. Of the two known classes of retinoid receptors, the retinoic acid receptors (RARα,β,γ)[2] and the retinoid X receptors (RXRα,β,γ),[3] the RXRs have received increased attention due to their ability to partner with other nuclear receptors as heterodimers. The natural ligand for the RXRs has been suggested to be 9-*cis*-retinoic acid (9-*cis*-RA)[4] and for the RARs it is all *trans*-retinoic acid (RA) (Figure 1). RXR ligands, such as 9-*cis*-RA, can activate gene transcription through RXR−RXR homodimers.[5] The ability of RXRs to form heterodimers with other receptors such as the peroxisomal proliferator activated receptors (PPARs) has established a central role for these proteins in many endocrine signaling pathways and suggests significant therapeutic applications for RXR ligands in the areas of lipid metabolism and diabetes. The observations that RXR ligands function as hypoglycemic agents in animal models of diabetes[6a] and inhibit cholesterol absorption[6b] have given additional impetus for development of compounds with complete RXR specificity. In this regard, we and others have reported on a series of RXR selective agents.[5,7] Here, we report on the enantioselective synthesis of the individual enantiomers of racemic compound **1** (Figure 1) which has been described previously.[7j] The *S,S* enantiomer, compound **2** (AGN 194204), is a potent and specific RXR agonist with nanomolar binding affinities to the RXRs and no detectable binding to the RARs (K_d >30K). In contrast, the *R,R* enantiomer, compound **3** (AGN 194277), is a 50−100-fold less potent RXR agonist with demonstrable RAR activity. We also demonstrate the potent hypoglycemic activity of **2** in an animal model of diabetes and correlate this effect with activation of RXRs.

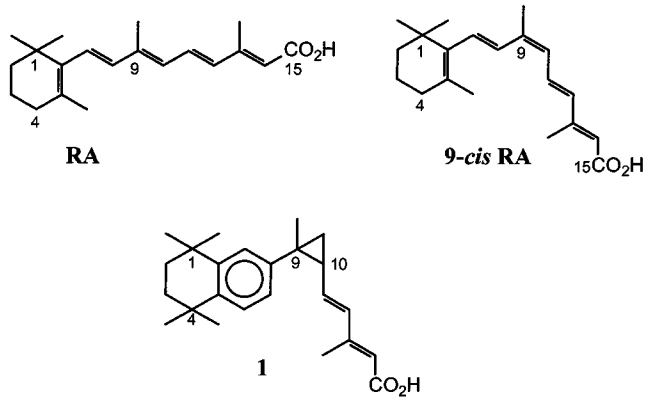

Figure 1.

Synthesis

The enantioselective syntheses of **2** and **3** are described in Scheme 1. Aryl bromide **4**[8] was reacted with 2-methyl-3-butyn-2-ol in the presence of Pd(II), and the resultant propargylic alcohol was treated with KOH in toluene to give the free acetylene **5** (87%). Compound **5** was converted to **6** by reaction with MeLi and ethyl chloroformate. Low temperature 1,4-addition of dimethylcopperlithium to **6** gave the *Z*-crotonate **7**. The α,β-unsaturated ester was reduced with DibAl-H to the *Z*-allylic alcohol **8** (68%). Enantioselective cyclopropanation[9] of **8** with (+)-diethyl tartarate gave the cyclopropyl alcohols **9** in 80% ee favoring the (−)-enantiomer. The mixture was converted to the diastereomeric camphenoate esters **10** and **11** by reaction with (−)-1-(*S*)-camphanic chloride in the presence of pyridine. The diastereomeric excess of the mixture was determined to be 80% de by HPLC. Diastereomers **10** and **11** were separated by HPLC, and the absolute stereochemistry of **10** was determined to be *S* and *R* at C-9 and C-10 (retinoid numbering), respectively, by single-crystal X-ray crystallography (Figure 2). The individual esters **10** and **11** were hydrolyzed to give enantiomerically pure

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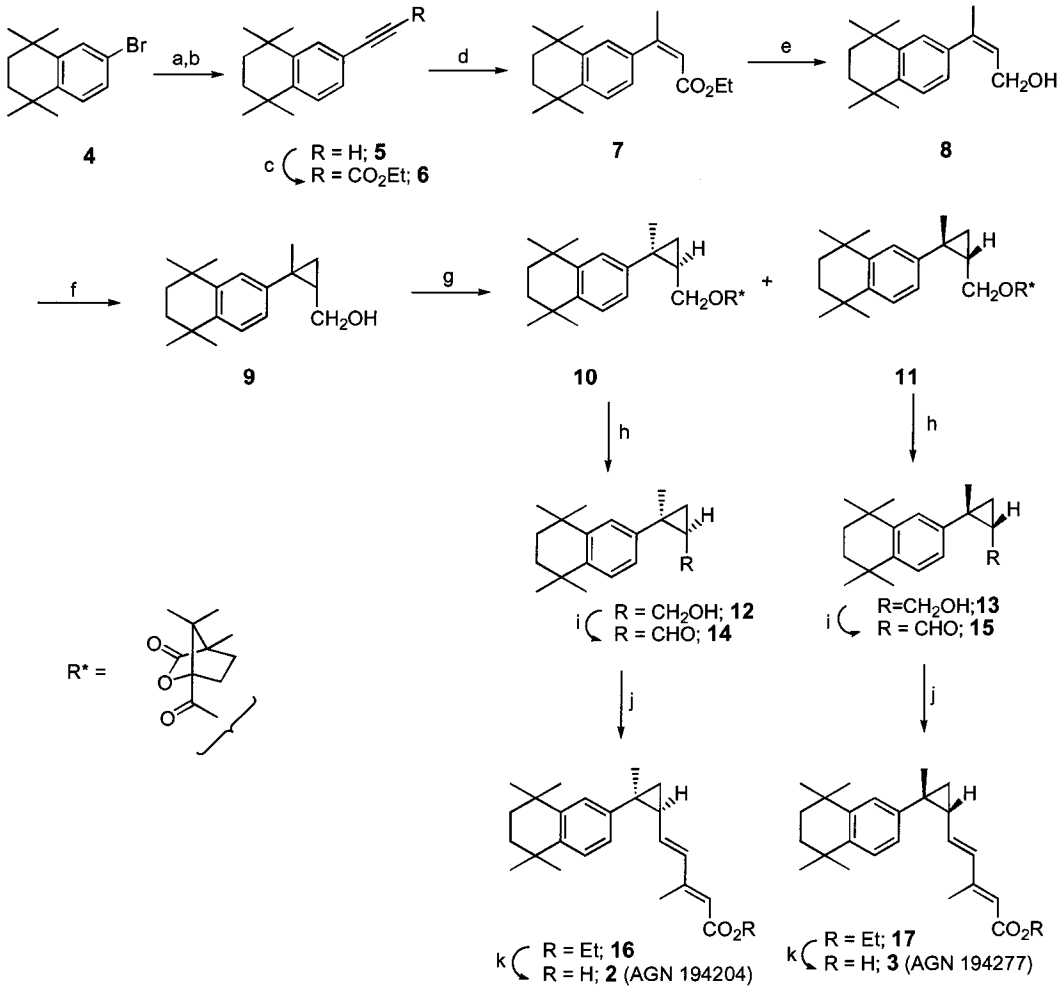
Scheme 1[a]



[a] Reagents and conditions: (a) 2-methyl-3-butyn-2-ol, $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, 75 °C, 85%; (b) KOH, toluene, 110 °C, 90%; (c) MeLi, $ClCO_2Et$, −10 °C, 65%; (d) Me_2CuLi, −78 °C, 90%; (e) DibAl-H, CH_2Cl_2, −78 °C, 95%; (f) Et_2Zn, CH_2I_2, L-(+)-diethyltartrate, −10 °C; (g) (−)-camphanic chloride, Py, 40 °C, 90%; HPLC separation; (h) KOH, H_2O, 90%; (i) NMO, TPAP, 90%; (j) diethyl (*E*)-3-ethoxycarbonyl-2-methylallylphosphonate, DMPU, *n*-BuLi, −78 °C, 90%; (k) KOH, H_2O, 80%.

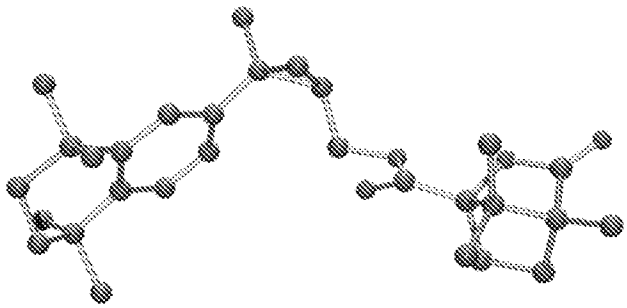


Figure 2. ORTEP diagram of camphenoate ester **10** (hydrogens have been omitted for clarity).

alcohols **12** and **13** and then oxidized to the corresponding aldehydes **14** and **15**[10] using *N*-methylmorpholine *N*-oxide (NMO) and tetrapropylammonium perruthenate (TPAP). Aldehydes **14** and **15** were reacted with diethyl (*E*)-3-ethoxycarbonyl-2-methylallylphosphonate in the presence of *n*-BuLi to give diene esters **16** and **17**, respectively, in 85% yield. In these Horner–Emmons reactions, less than 5% of the 13-*Z* isomers were formed, and the 13-*E* isomers, **16** and **17**, were separated by HPLC (in the larger scale synthesis of **2**, the compound was separated from its 13-*Z* impurity by recrystallization at the carboxylic acid stage). Hydrolysis of each ester, followed by recrystallization, gave the optically pure retinoid analogues **2** and **3**. On the basis of the *S*,*R* stereochemistry assigned to the camphenoate ester **10** by X-ray crystallography, *S*,*S* and *R*,*R* stereochemistries were assigned to **2** and **3**, respectively. The optical purity of compound **2** was established by repeat crystallization to obtain a constant specific rotation value.

Biological Evaluation

The binding affinities of the retinoid analogues were determined using baculovirus expressed receptors and competition with [^{3}H]-RA for the RARs and [^{3}H]-9-*cis*-RA for the RXRs.[11] The functional gene transcriptional activities of the retinoids were measured in transactivation assays using CV-1 cells transiently cotransfected with an individual receptor gene construct and a reporter gene.[12] These compounds were also tested in an animal model of diabetes for hypoglycemic activity.[6a,13]

Compound **2** with *S*,*S* stereochemistry at the C-9 and C-10 carbons is a very high affinity ligand for the RXRs with no detectable affinity to the RARs (Table 1). It binds with 10–30-fold higher affinity to the RXRs than 9-*cis*-RA. In contrast, compound **3** has 50–100-fold lower affinity for the RXRs than its antipode and also

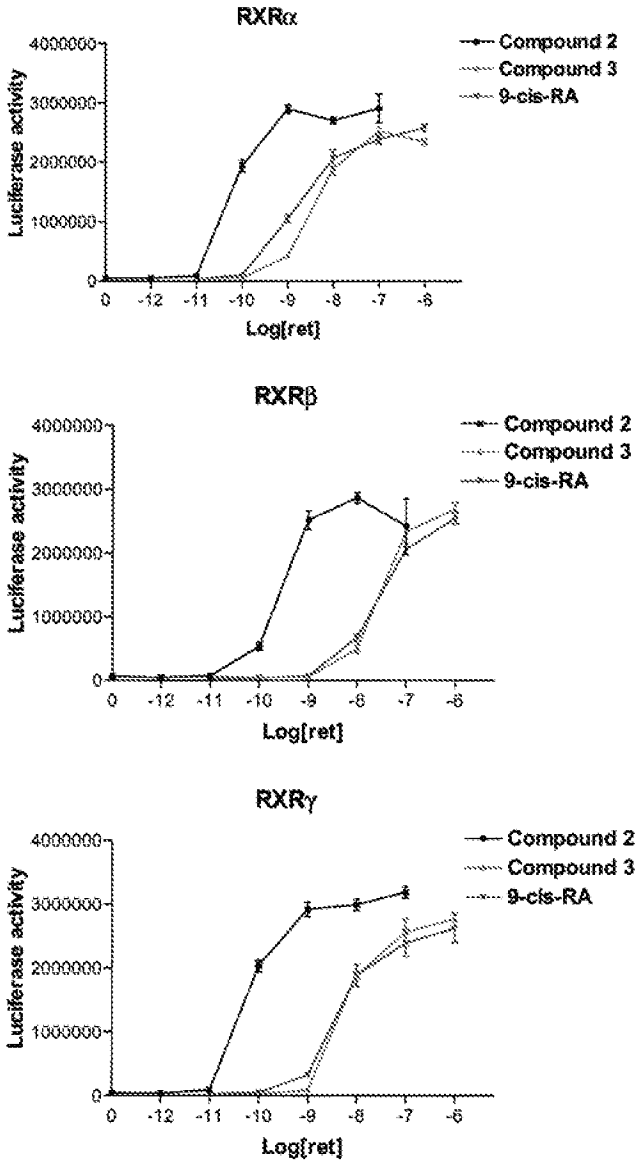


Figure 3. Dose response curves at each RXR for retinoids **2**, **3**, and 9-*cis* RA in CV-1 cells transfected with RXR holoreceptors and CRBPII-tk-Luc reporter plasmid.

Table 1. Receptor Binding Affinity (K_d, nM)

AGN no.	compd	RAR α	RAR β	RAR γ	RXR α	RXR β	RXR γ
	9-*cis* RA	90	100	150	13	35	30
	1	9500	20500	15900	1.5	2.5	1.8
194204	**2**	>30K	>30K	>30K	0.4	3.6	3.8
194277	**3**	5750	6200	>10K	60	210	180

Table 2. Transactivation Potency (EC_{50}, nM)

AGN no.	compd	RAR α	RAR β	RAR γ	RXR α	RXR β	RXR γ
	9-*cis* RA	21	3	4	1.5	29	4.5
194204	**2**	NA	NA	NA	0.2	0.8	0.08
194277	**3**	>500	>500	>500	4	25	6

binds very weakly to the RARs. The transcriptional potencies of compounds **2** and **3** correspond to their relative binding affinities (Figure 3 and Table 2). Thus, compound **2** is a potent activator of all three RXRs with EC_{50} values ranging from 0.08 nM (RXRγ) to 0.8 nM (RXRβ). Compound **3** is also an effective RXR agonist but is 20−80-fold less potent than **2**. Compound **2** is also 3−100-fold more potent than 9-*cis*-RA. Interestingly, compound **2** is somewhat selective for RXRγ and RXRα while 9-*cis*-RA is selective for RXRγ and RXRβ. This suggests that it might be possible to design pharmacologically useful RXR subtype-selective agonists despite the high degree of homology in the ligand binding domains of the three RXRs. Compound **2** is also an RXR specific agent since it failed to activate any transcription from the chimaeric RARs even at 1 μM (data not shown). In contrast, compound **3** showed weak activity at the RARs, particularly at RARγ (20−25% activation at 1 μM; data not shown).

Table 3. Hypoglycemic Effect of Carboxylic Acids **2** and **3** in *db*/*db* Mice

compd no.	dosage, mg/kg	animals/group	glucose, mg/dL (mean ± SD) day 0	glucose, mg/dL (mean ± SD) day 7
vehicle		10	402 ± 118	459 ± 154
2	0.4	10	406 ± 127	291 ± 112
2	2.0	10	404 ± 125	221 ± 42*
3	2.0	10	400 ± 110	391 ± 104
3	8.0	10	404 ± 125	441 ± 135

*$p < 0.02$, by ANOVA with Scheffe's post-hoc test, with respect to vehicle control.

The ability of these compounds to function as hypoglycemic agents was determined using the *db*/*db* mouse model which has been shown to be predictive of the clinical efficacy of anti-diabetic agents. The *db*/*db* mice are genetically defective in leptin signaling[14] and, as a consequence, become obese and hyperglycemic and develop insulin resistance. Compound **2** is a potent hypoglycemic agent (Table 3) in this animal model. After only 1 week of treatment, compound **2** at daily doses of 0.4 and 2 mg/kg lowered glucose levels by 37% and 53%, respectively, relative to control animals.[15] Compound **3** caused no significant reduction in serum glucose at a dose of 8 mg/kg. These results correlate activation of the RXR homodimer pathway with regulation of glucose levels in vivo. However, the ability of RXR ligands to exert hypoglycemic effects by activation of RXR−PPARγ or other RXR heterodimer pathways cannot be ruled out.

In conclusion, we have shown that the weak RAR activity associated with the racemic mixture **1** resides with its (−)-enantiomer, compound **3**, having the absolute configuration of *R*,*R* at the C-9 and C-10 carbons. Also, in compound **2** we have a potent RXR specific agent with no residual RAR activity. Compound **2** is the most potent RXR agonist described to date. The 9(*S*),10(*S*)-cyclopropyl moiety of **2** provides an ideal template for the design of potent and specific RXR panagonists and possibly RXR subtype-selective agonists as well as RXR antagonists. Finally, compound **2** displays potent hypoglycemic activity in the diabetic *db*/*db* mouse model.

Experimental Section

Melting points were determined using a Thomas-Hoover melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded using a Varian Gemini 300 spectrometer (300 MHz), and ^{13}C NMR spectra were recorded using a Varian Gemini 300 spectrometer (75 MHz) in the solvent indicated. Optical rotations were recorded on a Perkin-Elmer model 241 polarimeter. Elemental analyses were performed by Robertson

Microlit Laboratories, Inc., Madison, NJ. Thin-layer chromatography (TLC) was carried out using Whatman silica gel 60A plates (0.25 mm). Flash chromatography was performed using E. Merck silica gel 60 (230−400 mesh). All reactions were carried out under a positive pressure of argon using reagent grade or anhydrous solvents as received. In the compound preparations, use of the phrase "dried and evaporated" indicates that the compound solution was dried over $MgSO_4$ and the solvents were evaporated on a rotary evaporator under house vacuum.

Biological Methods. Receptor Transactivation Assay. The assay was performed as described by Klein et al.[16] Briefly, CV-1 cells were transfected with an expression vector for a given retinoid receptor and a luciferase reporter plasmid. Wild-type RXRs were used for the RXR homodimer transactivation assays. For RXR transactivation assays, the reporter plasmid CRBPII-tk-Luc which contains the RXR homodimer-binding sites from the rat cellular retinol binding protein II gene promoter was used. For RAR transactivation assays, the reporter plasmid ERE-tk-Luc, which contains the binding sites for estrogen receptors, was cotransfected with chimaeric ER-RARα, *β*, or *γ*, in which the DNA binding domain of RARs was substituted with that of the estrogen receptor. Eighteen hours after transfection, cells were rinsed with phosphate-buffered saline and fed with Dulbecco's modified Eagle's medium (Life Technologies, Inc.) containing 10% activated charcoal-extracted fetal bovine serum (Gemini Bio-Products). Cells were treated for 18 h with compounds at concentrations from 10^{-12} to 10^{-5} M. After rinsing with phosphate-buffered saline, cells were lysed and luciferase activity was measured. The EC_{50} values were calculated as the concentrations yielding 50% of the maximal luciferase activity at 10^{-5} M of each compound.

Ligand Binding Assay. Compounds were analyzed as described by Allegretto et al.[11c] for their ability to competitively inhibit specific binding of [^{3}H]ATRA or [^{3}H]9-*cis*-retinoic acid to baculovirus-expressed RARs and RXRs, respectively. Assays were run in the format of 96-deep-well plates in duplicates. Briefly, 5−50 μg total Sf9 cell extracts containing appropriate retinoid receptors were incubated (16−18 h at 4 °C) with 5 nM [^{3}H]ATRA (for RARs) or 10 nM [^{3}H]9-*cis*-retinoic acid (for RXRs) mixed with test compounds at concentrations from 10^{-12} to 10^{-4} M. Ligand-bound receptors were isolated using a hydroxylapatite (HAP) separation method. After wash, the HAP pellet was resuspended in scintillation fluid, and its radioactivity was measured using the 1450 Microbeta counter (Wallac Inc.). Dissociation constants (K_d) were calculated via application of the Cheng−Prussof algorithm.[11b]

In Vivo Assays. Seven week old hyperglycemic *db*/*db* mice were bled by the retro-orbital route and randomized into treatment groups based on serum glucose levels. Glucose levels were determined enzymatically by the glucose oxidase (Trinder) assay (Sigma, St. Louis). Animals received test compounds suspended in 5% PEG-400, 0.5% CMC 7LF, 0.05% Tween-80, and water daily for 7 days by oral gavage. Three hours after the last treatment, animals were bled a second time. All bleeds were performed on unfasted animals between 9 and 11 in the morning.

X-ray Structural Determination of Compound 10. A colorless crystal of approximate dimensions 0.15 × 0.24 × 0.40 mm was mounted on a glass fiber and transferred to a Siemens P4 diffractometer. The determination of Laue symmetry, crystal class, unit-cell parameters, and the crystal's orientation matrix were carried out according to standard procedures.[17] The intensity data were collected at 158 K using a $2\theta/\omega$ scan technique with Mo Kα radiation. The raw data were processed with a local version of CARESS which employs a modified version of the Lehman−Larsen algorithm to obtain intensities and standard deviations from the measured 96-step peak profiles. All 2481 data were corrected for Lorentz and polarization effects and were placed on an approximately absolute scale. The diffraction symmetry was 2/*m* with the systematic absence *hkl* for $h + k = 2n + 1$. The three possible monoclinic space groups are *C*2, *Cm*, and *C*2*m*. It was later determined that the noncentrosymmetric space group *C*2 was correct. All calculations were carried out using the SHELXTL program.[18] The analytical scattering factors for neutral atoms were used throughout the analysis. The structure was solved by direct methods and refined on $F2$ by full-matrix least-squares techniques. Atoms C(21), C(22), and C(26) were disordered. The assigned site occupancy factors were 0.70 for the major component and 0.30 for the minor component. Hydrogen atoms were included using a riding model. At convergence, $wR2 = 0.1091$ and GOF = 1.070 for 296 variables refined against all 2426 unique data (As a comparison for refinement on F, $R1 = 0.04455$ for those 2027 data with $F > 4.0\sigma(F)$.) It was not possible to assign the absolute structure by refinement of the Flack parameter or by inversion of the model.

Preparation of Acetylenic Compound 5. A solution of aryl bromide **4** (3.0 g, 11.3 mmol) in triethylamine (50 mL), CuI (100 mg, 0.55 mmol), $Pd(PPh_3)_2Cl_2$ (200 mg, 0.28 mmol), and 2-methyl-3-butyn-2-ol (3.5 g, 41.6 mmol) was prepared and argon bubbled through for 5 min. The mixture was then heated to 75 °C for 16 h. The reaction mixture was diluted with ether, and solids were filtered off. After evaporation of the solvent, the residue was purified by flash chromatography using 10% EtOAc in hexane as the eluent. The coupled product was isolated as a colorless oil. To this product were added toluene (75 mL) and KOH (1.5 g, 26.8 mmol), and the mixture was refluxed for 10 h. The mixture was diluted with ether (50 mL), washed with water (2 × 25 mL) and brine (30 mL), and then dried and evaporated. The acetylenic compound **5** was isolated as a colorless oil (2.1 g, 87%) by flash chromatography using 5% ethyl acetate in hexane. ^{1}H NMR ($CDCl_3$): δ 1.27 (s, 6 H), 1.31 (s, 6 H), 1.70 (s, 4 H), 3.01 (s, 1 H), 7.25 (brs, 2 H), 7.47 (s, 1 H).

Preparation of Propargyl Ester 6. To a cold (−78 °C) solution of compound **5** (4.2 g, 20 mmol) in THF (50 mL) was added MeLi in ether (1.4 M, 32 mL, 44.8 mmol) over a period of 5 min. The reaction was gradually warmed to −30 °C over 1 h and then was recooled to −78 °C, and neat ethylchloroformate (5.7 g, 53 mmol) was added via syringe. After 10 min, the reaction mixture was diluted with EtOAc (150 mL), washed with water (15 mL) and brine (15 mL), and then dried and evaporated. The crude product was purified by flash chromatography using 10% EtOAc in hexane to obtain pure propargyl ester **6** (4.3 g, 83%). ^{1}H NMR ($CDCl_3$): δ 1.27 (s, 12 H), 1.35 (t, J = 7.5 Hz, 3 H), 1.67 (s, 4 H), 4.30 (q, J = 7.5 Hz, 2 H), 7.28 (d, J = 8.1 Hz, 1 H), 7.32 (dd, J = 1.8, 8.1 Hz, 1 H), 7.55 (d, J = 1.8 Hz, 1 H).

Preparation of *Z*-α*β*-Unsaturated Ester 7. To a cold (−20 °C) mixture of CuBr·DMS (4.4 g, 22 mmol) in THF (40 mL) was added MeLi in ether (1.4 M, 31.4 mL, 44 mmol) dropwise over a period of 10 min. The mixture was stirred for 10 min and cooled to −78 °C. To this cold dimethylcopper-lithium mixture was added propargyl ester **6** (4.3 g, 15.1 mmol) dropwise via syringe over a period of 20 min. The reaction was stirred at −78 °C for 4 h and quenched by dropwise addition of ethanol (5 mL) at −78 °C. The mixture was stirred at −78 °C for an additional 40 min. Water (6 mL) was then added, and the mixture was gradually warmed to −10 °C (2 h). The reaction was diluted with EtOAc (200 mL), washed with water, and filtered. The filtrate was washed with 10% HCl, water, 10% $NaHCO_3$, and brine, and it was then dried and evaporated. After flash chromatography with 5% EtOAc in hexane, compound **7** was isolated as a colorless syrup (4.1 g, 90%). ^{1}H NMR ($CDCl_3$): δ 1.03 (t, J = 7.5 Hz, 3 H), 1.25 (s, 6 H), 1.28 (s, 6 H), 1.67 (s, 4 H), 2.20 (s, 3 H), 3.97 (q, J = 7.5 Hz, 2 H), 5.85 (s, 1 H), 6.97 (dd, J = 1.9, 8.0 Hz, 1 H), 7.15 (d, J = 1.9 Hz, 1 H), 7.24 (d, J = 8.0 Hz, 1 H).

Preparation of *Z*-Allylic Alcohol 8. To a cold (−78 °C) solution of compound **7** (4.1 g, 13.3 mmol) in dichloromethane (30 mL) was added DibAl-H in dichloromethane (1 M, 32 mL, 32 mmol). The reaction mixture was gradually warmed to 0 °C (2 h) and quenched by the addition of MeOH (4 mL), followed by water (10 mL) and 10% HCl (10 mL). The mixture was stirred at 0 °C for 10 min. The organic layer was separated, washed with brine, dried, and evaporated. Flash chromatography with 10% EtOAc in hexane gave the Z-allylic

alcohol **8** (2.4 g, 68%). ^{1}H NMR ($CDCl_3$): δ 1.28 (s, 6 H), 1.31 (s, 6 H), 1.70 (s, 4 H), 2.24 (s, 3 H), 4.14 (t, J = 6.3 Hz, 2 H), 5.69 (t, J = 6.3 Hz, 1 H), 6.96 (dd, J = 1.8, 8.1 Hz, 1 H), 7.09 (d, J = 1.8 Hz, 1 H), 7.26 (d, J = 8.1 Hz, 1 H).

Camphenoate Esters (Diastereomers 10 and 11). To a cold (0 °C) solution of *Z*-allylic alcohol **8** (920 mg, 3.6 mmol) in dichloromethane (18 mL) was added diethylzinc (1 M solution in heptane, 4 mL, 4 mmol), and the solution was stirred for 15 min. To this solution was added neat l-(+)-diethyl tartrate (806 mg, 3.96 mmol) dropwise via syringe over a period of 3 min. The mixture was stirred for 1 h and then cooled to −25 °C, and another portion of diethylzinc in heptane (1 M solution, 8 mL) was added. Stirring was continued for an additional 10 min. To this mixture was added neat diiodomethane (3.96 g, 14.9 mmol). The reaction was stirred at −4 °C for 4 days and then was poured into aqueous NH_4Cl solution and diluted with ether (120 mL), washed with 10% HCl, $NaHCO_3$, and brine, and then was dried and evaporated. The mixture was purified by flash chromatography using 10% EtOAc in hexane to obtain the enantiomeric cyclopropyl alcohols **9**. These purified enantiomeric cyclopropyl alcohols were dissolved in dichloromethane (10 mL), pyridine (5 mL), and *N*,*N*-(dimethylamino)pyridine (50 mg) and cooled to 0 °C. To this mixture was added 1-(*S*)-(−) camphanic chloride (1.57 g, 7.2 mmol), and the mixture was heated at 40 °C overnight. After cooling to ambient temperature, the mixture was diluted with ether (60 mL), washed with water (10 mL), 10% HCl (20 mL), and brine (10 mL), and then was dried and evaporated. The material was passed through a flash column using 5% EtOAc in hexane to obtain the two diastereomers in a 10:1 ratio. The major diastereomer **10** was recrystallized from hexane. The single-crystal X-ray crystallographic structure revealed it to have 9 *S* and 10 *R* (retinoid numbering) absolute stereochemistry. The minor diastereomer **11** was purified by HPLC (Whatman, Partisil-10-PAC preparative HPLC column) using 8% EtOAc in hexane (RT of 128 min for the major diastereomer **10**, and 137 min for the minor diastereomer **11**).

Diastereomer 10. $[\alpha]^{21}_D$: −10.7 (*c*, 0.005, CH_2Cl_2). Mp: 126−127 °C. ^{1}H NMR ($CDCl_3$): δ 0.82−0.90 (m, 1 H), 0.90−0.96 (m, 1 H), 0.96 (s, 3 H), 1.04 (s, 3 H), 1.22 (s, 3 H), 1.25 (s, 3 H), 1.26 (s, 3 H), 1.27 (s, 3 H), 1.28 (s, 3 H), 1.28−1.40 (m, 1 H), 1.38 (s, 3 H), 1.62−1.75 (m, 1 H), 1.66 (s, 4 H), 1.85−2.05 (m, 2 H), 2.30−2.42 (m, 1 H), 3.74 (dd, J = 11.5, 7.7 Hz, 1 H), 3.98 (dd, J = 11.5, 7.7 Hz, 1 H), 7.04 (dd, J = 2.0, 8.0 Hz, 1 H), 7.18 (s, 1 H), 7.19 (d, J = 8.0 Hz, 1 H).

Diastereomer 11. $[\alpha]^{21}_D$: −5.4 (*c*, 0.007, CH_2Cl_2). Mp: 65−68 °C. ^{1}H NMR ($CDCl_3$): δ 0.82−0.89 (m, 1 H), 0.91−0.98 (m, 1 H), 0.94 (s, 3 H), 1.06 (s, 3 H), 1.12 (s, 3 H), 1.24 (s, 3 H), 1.26 (s, 3 H), 1.26 (s, 3 H), 1.27 (s, 3 H), 1.28−1.40 (m, 1 H), 1.39 (s, 3 H), 1.65−1.73 (m, 1 H), 1.67 (s, 4 H), 1.85−2.05 (m, 2 H), 2.35−2.45 (m, 1 H), 3.76 (dd, J = 11.5, 7.7 Hz, 1 H), 3.95 (dd, J = 11.5, 7.7 Hz, 1 H), 7.04 (dd, J = 2.0, 8.0 Hz, 1 H), 7.18 (s, 1 H), 7.19 (d, J = 8.0 Hz, 1 H).

Preparation of Enantiomerically Pure (−)-Cyclopropyl Alcohol 12. Diastereomer **10** (240 mg, 0.53 mmol) was dissolved in THF (2 mL), MeOH (2 mL), and LiOH−H_2O (1 M solution, 1.5 mL) and was heated to 70 °C for 1 h. The reaction mixture was diluted with water (5 mL) and extracted with ether (3 × 30 mL). The organic layer was washed with water (10 mL) and brine (10 mL) and then was dried and evaporated. The crude material was purified by flash chromatography using 10% EtOAc in hexane as the eluent to give the cyclopropyl alcohol **12** (135 mg, 93%) as a colorless oil. $[\alpha]^{21}_D$: −22.7 (*c*, 0.023, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 0.76 (dd, J = 8.4, 4.8 Hz, 1 H), 0.87 (t, J = 4.8 Hz, 1 H), 1.27 (s, 6 H), 1.28 (s, 6 H), 1.25−1.31 (m, 1 H), 1.40 (s, 3 H), 1.67 (s, 4 H), 3.18 (dd, J = 11.6, 7.9 Hz, 1 H), 3.31 (dd, J = 11.6, 7.9 Hz, 1 H), 7.07 (dd, J = 2.0, 8.0 Hz, 1 H), 7.22 (d, J = 8.0 Hz, 1 H), 7.23 (s 1 H).

Preparation of (+)-Cyclopropyl Aldehyde 14. To a solution of cyclopropyl alcohol **12** (135 mg, 0.5 mmol) in dichloromethane (5 mL) was added NMO (245 mg, 2.01 mmol) and TPAP (5 mg). The mixture was stirred for 45 min and then was passed through a small of column of silica gel and eluted with 5% EtOAc in hexane. Cyclopropyl aldehyde **14** was isolated as a colorless oil (130 mg, 96%). This aldehyde is not very stable for storage and was used immediately after it was prepared. $[\alpha]^{21}_D$: +15.9° (*c*, 0.023, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 1.23 (s, 3 H), 1.24 (s, 6 H), 1.27 (s, 3 H), 1.45 (s, 3 H), 1.35−1.45 (m, 1 H), 1.67 (s, 4 H), 1.80−2.00 (m, 2 H), 7.11 (dd, J = 2.0, 8.2 Hz, 1 H), 7.27 (d, J = 8.2 Hz, 1 H), 7.30 (d, J = 2.0 Hz, 1 H), 8.34 (d, J = 6.8 Hz, 1 H).

Preparation of (+)-Ethyl Ester 16. To a cold (−78 °C) solution of triethylphosphono-3-methyl-2(*E*)-butenoate (528 mg, 2 mmol) in THF (5 mL) and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU) (1.5 mL) was added *n*-BuLi in hexane (1.6 M solution, 1.3 mL, 2.0 mmol). The mixture was stirred for 2 min. To this reaction mixture was added a solution of cyclopropyl aldehyde **14** (125 mg, 0.46 mmol) in THF (2 mL) at −78 °C, and the mixture was stirred for 1 h. The reaction was diluted with water (10 mL), extracted with ether (3 × 40 mL), washed with water (10 mL) and brine (10 mL), and then was dried and evaporated. The product was purified by column chromatography (using 5% EtOAc in hexane), followed by HPLC separation (partisil-10-PAC, semi-prep column, using 1% EtOAc in hexane) to give compound **16** as a colorless oil (149 mg, 85%). $[\alpha]^{21}_D$ +82.33° (*c*, 0.014, CH_2Cl_2). ^{1}H NMR ($CDCl_3$): δ 1.11−1.20 (m, 2 H), 1.21 (s, 3 H), 1.28 (s, 9 H), 1.28 (t, J = 7.2 Hz, 3 H), 1.43 (s, 3 H), 1.68, (s, 4 H), 1.68−1.80 (m, 1 H), 1.99 (s, 3 H), 4.15 (q, J = 7.2 Hz, 2 H), 5.22 (dd, J = 9.9, 15.4 Hz, 1 H), 5.63 (s, 1 H), 6.20 (d, J = 15.4 Hz, 1 H), 7.03 (dd, J = 1.9, 8.1 Hz, 1 H), 7.14 (d, J = 1.9 Hz, 1 H), 7.23 (d, J = 8.1 Hz, 1 H). ^{13}C NMR ($CDCl_3$): ppm 13.53, 14.34, 22.04, 28.72, 29.72, 30.39,31.79, 31.83, 31.99, 33.98, 34.19, 35.11, 35.14, 59.45, 116.19, 126.28, 126.50, 127.52, 131.42, 139.69, 140.24, 142.76, 144.51, 152.68, 167.43.

Preparation of Compound 2 (AGN 194204). To a solution of **16** (29 mg, 0.08 mmol) in THF (2 mL) and MeOH (2 mL) was added NaOH (1 M solution, 1 mL), and the mixture was stirred for 24 h at ambient temperature. The mixture was diluted with ether (50 mL), acidified with 10% HCl, washed with water (5 mL) and brine (5 mL), and then was dried and evaporated. The crude material was purified by flash chromatography using 25% EtOAc in hexane to afford compound **2** as a white solid (21 mg, 78%). $[\alpha]^{21}_D$: +86.30 (*c*, 0.0015, CH_2Cl_2). Mp: 137−138 °C. ^{1}H NMR ($CDCl_3$): δ 1.14−1.20 (m, 2 H), 1.20 (s, 3 H), 1.27 (s, 9 H), 1.42 (s, 3 H), 1.66, (s, 4 H), 1.68−1.80 (m, 1 H), 1.98 (s, 3 H), 5.27 (dd, J = 9.9, 15.4 Hz, 1 H), 5.64 (s, 1 H), 6.21 (d, J = 15.4 Hz, 1 H), 7.03 (dd, J = 1.9, 8.1 Hz, 1 H), 7.12 (d, J = 1.9 Hz, 1 H), 7.24 (d, J = 8.1 Hz, 1 H). ^{13}C NMR ($CDCl_3$): ppm 13.75, 22.19, 28.67, 29.82, 30.67, 31.80, 31.84, 31.97, 33.99, 34.19, 35.18, 115.40, 126.31, 126.48, 127.53, 131.33, 139.63, 141.47, 142.85, 144.57, 155.17, 172.54. Anal. Calcd for $C_{24}H_{32}O_2$: C, H.

Preparation of Enantiomerically Pure (+)-Cyclopropyl Alcohol 13. Employing the procedure used for the preparation of compound **12**, compound **13** was prepared from diastereomer **11** (yield 90%). $[\alpha]^{21}_D$: +21.8 (*c*, 0.02, CH_2Cl_2). Calculated optical purity is 96%. Compound **13** is identical to compound **12** by ^{1}H and ^{13}C NMR analyses.

Preparation of (−)-Cyclopropyl Aldehyde 15. Employing the procedure used for the preparation of compound **14**, compound **15** was prepared from **13** (yield 90%). $[\alpha]^{21}_D$: −13.6° (*c*, 0.005, CH_2Cl_2). Calculated optical purity is 86%. The low calculated optical purity of the aldehyde **15** is likely due to the instability of the compound. Compound **15** is identical to compound **14** by ^{1}H NMR analysis.

Preparation of (−)-Ethyl Ester 17. Employing the procedure used for the preparation of compound **16**, compound **17** was prepared from **15** (yield 85%). $[\alpha]^{21}_D$: −80.8° (*c*, 0.0025, CH_2Cl_2). Calculated optical purity is 98%. Compound **17** is identical to compound **16** by ^{1}H and ^{13}C NMR analyses.

Preparation of (−)-Compound 3 (AGN 194277). Employing the procedure used for the preparation of compound **2**, compound **3** was prepared from **17** (yield 80%). $[\alpha]^{21}_D$: −84.3° (*c*, 0.0015, CH_2Cl_2). Calculated optical purity is 98%. Compound **3** is identical to compound **2** by ^{1}H and ^{13}C NMR analyses. Mp: 137−138 °C. Anal. Calcd for $C_{24}H_{32}O_2$: C, H.

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